

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
<p>Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.</p>				
1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE 01/29/97	3. REPORT TYPE AND DATES COVERED Final 01 June 1993 - 31 May 1996		
4. TITLE AND SUBTITLE Molecular Marine Biology Research Training		5. FUNDING NUMBERS G N0014-93-1-0901		
6. AUTHOR(S) Epel, David		7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Hopkins Marine Station of Stanford University Oceanview Blvd Pacific Grove CA 93950		
8. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) na		9. PERFORMING ORGANIZATION REPORT NUMBER na		
10. SPONSORING / MONITORING AGENCY REPORT NUMBER na		11. SUPPLEMENTARY NOTES na		
12. DISTRIBUTION / AVAILABILITY STATEMENT Available to the public				
13. ABSTRACT (Maximum 200 words) This grant provided training in developmental biology of embryos of marine organisms, particularly their defense mechanisms. Students were exposed to the patterns of development of a variety of marine organisms, and then trained in the use of cellular, immunological and molecular approaches for studying these embryos and their defense mechanisms. We focused on the defense mechanisms of embryos since this is a poorly studied but important area. Thus we understand a lot about adult defenses, but little about how these develop and more importantly whether the mechanisms in early embryos are different than those of the adult. Also, knowing how the embryo defends itself is critical to predicting the effects of environmental stress, since the embryonic phases are the most vulnerable part of the life history of the organism. The training also included a brief research project, which provided the students an opportunity to use their newly-acquired skills to attain new knowledge about these defense mechanisms. In conclusion, molecular and cellular information about this critical stage in the life history of the organism is lacking, and the training in this new research area will thus importantly impact the marine sciences				
14. SUBJECT TERMS molecular biology, developmental biology, larval biology, environmental stress, graduate training		15. NUMBER OF PAGES 9		
16. SECURITY CLASSIFICATION OF THIS PAGE na		17. PRICE CODE na		
17. SECURITY CLASSIFICATION OF REPORT na	18. SECURITY CLASSIFICATION OF THIS PAGE na	19. SECURITY CLASSIFICATION OF ABSTRACT na	20. LIMITATION OF ABSTRACT UL	

19970203 004

Distribution List for Annual and Final Reports

1. Put a cover page (Form DD 1473) on your report and attach a copy of the distribution list. Mail one copy of the report to each person on the contractor subset list attached on which your name appears. The other subset list is for your information only. Please don't forget to attach this distribution list to your report - otherwise the folks below think they have mistakenly received the copy meant for the Molecular Biology Program and forward it to us.

2. Mail two copies to (include a DTIC Form 50 with these two copies too)

~~Administrator~~

~~Defense Technical Information Center
Building 5, Cameron Station
Alexandria, VA 22314~~

Defense Technical Information Center
8725 John J Kingman Road, Suite 0944
Ft. Belvoir VA 22060-6218

3. Mail one copy to each of the following:

(a) Dr. Michael Marron
ONR Code 1141
Molecular Biology Program
800 N. Quincy Street
Arlington, VA 22217-5000

616-4038

(b) Administrative Contracting Officer
ONR Resident Representative
(address varies - see copy of your grant)
see below

(c) Director,
Applied Research Directorate
ONR Code 12
800 N. Quincy Street
Arlington, VA 22217-5000

(d) Director
Office of Naval Technology
Code 22
800 N. Quincy Street
Arlington, VA 22217-5000

(b) Linden Clausen
ONR Seattle Regional Office
1107 NE 45th Street, Suite 350
Seattle WA 98105-4631

(e) Director
Chemical and Biological Sci Div
Army Research Office
P. O. Box 12211
Research Triangle Park, NC 27709

(f) Life Sciences Directorate
Air Force Office of Scientific Research
Bolling Air Force Base
Washington, DC 20332

(g) Director
Naval Research Laboratory
Technical Information Div, Code 2627
Washington, DC 20375

FINAL REPORT ON ONR GRANT
N00014-93-1-0901
Molecular Marine Biology Research Training
June 1, 1993-May 31, 1996
David Epel,
Hopkins Marine Station of Stanford University
Principal Investigator

The objective of this program was to expose and redirect young scientists at the graduate, postdoctoral and advanced undergraduate level to a new and important area in marine biology, that of how embryos of marine organisms cope with stress; to provide participants with training in cell and molecular techniques in this area and to expose them to the research process in this field by having them design and complete an original research project in this area.

The training program focused on how the embryonic and larval life stages, undoubtedly the most sensitive phase of the life history of the organism, cope with environmental stress. The program consisted of three parts. The first centered on instruction and lab exposure to the developmental biology of selected invertebrate embryos that can be studied easily in the lab and serve as model organisms, such as tunicate, sea urchin, starfish and fish embryos. This included microscopy and immunofluorescence approaches. The second part of the course focused on the cell and molecular biology of various environmental stresses such as ultraviolet radiation, temperature, pollutants, pathogens and stress proteins. The third component centered on short research projects suggested by the lab observations made during the earlier part of the course.

Twenty-five students participated in the program over its three year duration. The students were of diverse backgrounds, and included 15 graduate students and 10 undergraduates. We have not kept up contact with all the undergraduates, but know that five of the ten are now graduate students or will be graduate students in areas related to marine biology. Of the 25 students, 14 were female and 11 were male. There was one minority student (Hispanic). There were also 4 foreign students, including participants from Canada, Germany, Chile and Venezuela.

The program brought many guest lecturers to the Marine Station, which also benefited general graduate education at the Hopkins Marine Station, since the general student body also attended these lectures. The list of guest lecturers (as a syllabus listing the schedule of classes for the three years) is attached.

The students also became involved in original research projects as part of the course, and the results of their research was presented in the form of a symposium on the last day of the course. A listing of these research projects is attached (labeled the China Point Academy of Sciences Program, in recognition of one of the synonyms for the location of the Hopkins Marine Station).

These research projects deserve some comment in this final report. Although carried out over only a two-week period, they are highly focused projects based on original observations made in the lab during the first three weeks of the course. These projects arouse intense interest of the students, and often have impacts far beyond the five weeks of the course.

Below I will provide several examples of these impacts over the current three years of this grant period. Nikki Adams worked in 1994 on UV effects in sea urchin embryos, and the techniques and insights she gained in the course and project are forming an important part of her Ph.D. thesis in Malcolm Shicks' lab at the University of Maine, where she is working on UV protective compounds in sea urchin embryos. Another student is Scott Shofer, a graduate student in chemical toxicology at UC Santa Cruz, who obtained an appreciation of larval biology which has become an important adjunct to his training in classical chemistry. There is also an important impact on undergraduates who take this course, as this intense summer experience redirects them to graduate studies in marine sciences. A particular example is Silvio Armitano, who will be a graduate student at Scripps Institution of Oceanography.

These research projects often provide novel insights that can later lead to publication of scientific reports. Two of particular relevance are the work of Brendan Flannery, whose project led to the preparation of a major paper on sperm receptors in tunicate eggs and the work of Katherine Hemela, whose project led to a major discovery of UV shields in the outer cells of the tunicate embryo (this work is being prepared for publication).

In summary, these summer training workshops have had a major impact in training and exposing young scientists at critical points in their career to new areas of study in marine biology. These workshops have affected their current work, as well as redirected some of them to new areas of research.

LECTURE SCHEDULE
CELL BIOLOGY OF EARLY DEVELOPMENT
EMBRYO DEFENSE MECHANISMS
SUMMER 1993

Week 1-June 14 to June 18

June 14-Overview of course / development of urechis and David Epel starfish-

June 15-sea urchin and sand dollar development-D. Epel

June 16-continuation of echinoids-D. Epel

June 17-Tunicates (and report on experiments with echinoids)- D. Epel

June 18-Medaka-Joel Sohn, Hopkins Marine Station

Week 2-June 21 to 25

June 21-24: The cell cycle/cell motility-Daniel Mazia (Stanford), Jerry Schatten and Cal Simerly (Wisconsin).

June 25- Stress response-Paul Levine (Stanford)

June 26-Stanford Youth Medical Science Program

Week 3-June 28 to July 2

June 28-Ultraviolet damage-Allan Smith (Stanford)

June 29-Xenobiotics-D.Epel, Francois Galgani(Nantes, France) and Roger Cornwall (Stanford)

June 30-Xenobiotics-Gary Cherr (Bodega Bay Marine Lab)

July 1-Pathogens/Symbiosis-Margaret McFall-Ngai (University of Southern California)/Paul Levine (Stanford).

July 2-Cell injury-Richard Steinhardt (Berkeley)

July 2-Planning for Research Projects

Weeks 4 and 5-Research Projects

July 17-Symposium on projects

CHINA POINT ACADEMY OF SCIENCES SYMPOSIUM

Summer 1993

(Research projects from "*Biomechanics*" and "*Cell Biology*" Courses)

Boat Works Auditorium
Friday, July 16

8:30	Ben Hale	Mechanical consequences of grazing scars produced by two intertidal limpets on the brown alga <i>Egregia</i>
8:45	Mats Lindegarth	Turbulent diffusion of larvae near complex shorelines
9:00	Jay Weems	Effects of micro-injected cyclin on sea urchin eggs (and the lack thereof)
9:15	Susan Chandra	Arrest of cortical reaction by heat in <i>Lytechinus pictus</i>
9:30	Ann Gibbons	Antioxidant protection at fertilization in <i>Lytechinus pictus</i>
9:45	Marco Raineri	Cytological effects of UV irradiation in early stages of development in sea urchins
10:00	COFFEE BREAK	
10:30	Suzanne Peyer	Material properties and drag on a corraline alga, <i>Calliosthron</i> sp.
10:45	Serge Lamarche	Ca^{+2} and heat shock in neuroblastoma cells
11:00	Lin Yip	Differentiation without cleavage in <i>Urechis caupo</i>
11:15	Melissa Foltz	The effects of quinacrine, a phospholipase A_2 inhibitor, on the fertilization and development of <i>Dendraster excentricus</i> .
11:30	John Kim	Effects of paper mill effluent on fertilization in tunicates

LECTURE SCHEDULE
CELL BIOLOGY OF EARLY DEVELOPMENT:
DEFENSE MECHANISMS OF EMBRYOS
SUMMER 1994

WEEK 1-EMBRYOLOGY/DEVELOPMENTAL BIOLOGY

July 18-Introduction to course

-Overview of development-Epel(HMS)

19-Sea urchin development -Ruediger (HMS/Univ S. Florida)

20-Spiralian development-Barbara Boyer (Union College)

21-Activation of the egg-Epel (HMS)

22-Metabolism of development-Barney Rees(HMS)

WEEK 2-CELL BIOLOGY /STRESS AND DEFENSES

July 25-Cell injury and repair-Richard Steinhardt-UC Berkeley

26-UV damage and repair-Philip Hanawalt-Stanford

27-Symbiosis and pathogens-Melissa Kaufman(HMS)

28-Stress by embryo culture-John Biggers (Harvard)

4:00 pm-Station seminar by R. DiGiulio

29-Stress proteins-Paul Levine (HMS)

-Oxidative stress-Richard DiGiulio (Duke)

WEEK 3-CELL BIOLOGY/CYTOSKELETON & EGG ACTIVATION
/POLLUTION DEFENSES

Aug 1-3---Special module on the cytoskeleton, involving
immunocytochemistry lectures and lab led by Daniel Mazia (HMS)
and Gerald Schatten (Wisconsin)

1-Cytoskeleton/Fertilization-Gerald Schatten(Wisconsin)

2-Parthenogenesis-Daniel Mazia (HMS)

3-Cytoskeleton/Fertilization (ctd) Gerald Schatten(Wisconsin)

4-Pollutants and the cell cycle-Gary Cherr (Davis)

5-Multixenobiotic transporters-Barbara Toomey (HMS)

WEEKS 4 AND 5--RESEARCH PROJECTS

August 19--**China Point Academy of Sciences** presentation of
research results from projects

**CHINA POINT ACADEMY OF SCIENCES
CELL BIOLOGY OF EARLY DEVELOPMENT:
EMBRYO DEFENSE MECHANISMS**

9:00 AM, FRIDAY, AUGUST 19
BOAT WORKS LECTURE HALL

9:00 -Dennis Powers, Director of Hopkins Marine Station
Welcome and introductory remarks

9:10-Nikki Adams
Intracellular targets of UV-induced cleavage delay in sea urchin embryos

9:25-Ned Clark
The effects of heat stress on the cell cycle in fertilized *L. pictus* embryos: An immunocytochemical study

9:40-Marc Kramer
Effects of heat stress on multixenobiotic pump activity in *Urechis caupo* embryos

9:55-Scott Shofer
Chemical transformation of pentachlorophenol in larvae of *Urechis caupo* in the presence and absence of multixenobiotic transport activity

10:10-Sabino Herrera
Regulation of the multixenobiotic pump in *Urechis* eggs

10:25-Caren Braby
Loligo opalescens : Accessory nidamental glands and bacteria in hatchling?

10:40-Jennifer Kreshak
Does *Shewanella putrefaciens*, the symbiotic bacterium of the squid egg casing, deter predators?

Lori Moilanen-(presented in absentia)
Probing reactive oxygen species in *Lytechinus pictus* sperm: art and artifact

10:55-COFFEE AND DONUTS

REVISED LECTURE SCHEDULE
CELL BIOLOGY OF EARLY DEVELOPMENT:
EMBRYO DEFENSE MECHANISMS
1995

(all lectures at 9:00 am in Agassiz 11)

WEEK 1

July 24

D. Epel(Stanford) Introduction to course
 Overview of development

July 25

D. Epel Activation of development

July 26

P. Hanawalt(Stanford) UV damage and repair
P. Tyler (Southampton, U.K.) Reproduction in the deep sea
(special lecture, 3:00 pm, MBARI lecture room)

July 27

R. Steinhardt(Berkeley) Cell membrane damage and repair

July 28

K. Mead(Stanford) Turbulence and fertilization

WEEK 2

July 31

M. Kaufman(Stanford) Symbiotic bacteria and embryos

August 1

D. Manahan (USC) Environmental physiology of
marine invertebrate development

August 2

G. Cherr(Davis) Xenobiotics-Cellular response of
embryos

August 3

B. Toomey(Duke) Xenobiotics-Defense mechanisms
of embryos

August 4

M. Hoshi(Tokyo)

Maturation of starfish oocytes

WEEK 3

August 6

ISIR Symposium on Fertilization-UCSC (all day)

August 7

ISIR Symposium on Fertilization-UCSC (all day)

August 8

ISIR Symposium on Embryo Defense Mechanisms-UCSC (am)

S. Lindquist(Chicago)

Heat shock proteins in embryos-
Earth & Marine Sciences Bldg,
UCSC (Rm A340), 2-4 pm

August 9

D. Harvell(Cornell) and
N. Lindquist(N. Carolina)

Chemical defenses of embryos

August 10

D. Mazia(Stanford)

Cell organization

August 11

G. Schatten(Wisconsin) The centrosome in fertilization
and cell organization

WEEK 4 AND 5

RESEARCH PROJECTS

August 25

Research symposium on results of projects

**Annual China Point Academy of Sciences
Hopkins Marine Station
Friday August 25, 1995**

9:00 - 9:05 a.m.	Opening Remarks	Dr. Dennis Powers, Director Hopkins Marine Station
------------------	-----------------	---

DEFENSE MECHANISMS

9:05 - 9:20	Bacterial Interaction With the Egg Surface of the Sea Urchin: Specific or Non - Specific?	Lisa Cameron
9:20 - 9:35	Do Sea Urchin Eggs Produce Anti - Fungal or Anti - Bacterial Compounds?	Prashant Shah
9:35 - 9:50	Mmm....Good. Marine Egg, Embryo, and Larva Palatability: Chemical Defenses During Development	Meredith Fisher

STRESS AND DEVELOPMENT

9:50 - 10:05	Does the Fertilization Membrane Confer Toxin Resistance to the Embryo?	Nancy Eufemia
10:05 - 10:20	The Effects of Salinity on the Development of <i>Dendraster excentricus</i>	Marianne Donahue

COFFEE BREAK

10:45 - 11:00	A Window of Thermotolerance During Early Development	Silvia Armitano
---------------	---	-----------------

FERTILIZATION AND NORMAL DEVELOPMENT

11:00 - 11:15	A Comparison of Normal Vs. Precocious Micromere Formation Using Time - Lapse Video Microscopy and Immunocytochemistry	Jennifer Hoffman
11:15 - 11:30	The Effects of Soybean Trypsin Inhibitor and Low Sodium Seawater on Fertilization In the Sea Urchin	Frank Sperling
11:30 - 11:45	Interspecific Gamete Interaction: Effects of Gametes From Non - Congeneric Species on Fertilization of the Sea Urchin, <i>Lytechinus pictus</i>	Patrick Leong